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# AN INQUIRY INTO THE CAUSES FOR VARIATION IN DETERMINATIONS OF DISINFECTING VALUE \*

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Having noted discrepancies in results of experiments for the determination of the disinfecting value of chemicals, especially in tests made on different days, we attempted to determine the reason for these variations. Difference in number of organisms in the culture on different days was the first thing that suggested itself as a cause. We therefore purposely varied, to a considerable degree, the number of organisms added to the water prior to disinfection.

*Technic.*—A 24-hour typhoid culture grown in standard broth of a reaction of +1.5 acid to phenolphthalein was filtered through paper and, in different experiments, quantities varying from 0.1 ml. to 3 ml. were discharged into 75 ml. of water contained in wide-mouthed sterile glass-stoppered bottles. Samples from each of these were taken for counting the number of bacteria by the usual technic. The infected bottles were brought to a temperature of 20 C. and kept at this temperature by means of a water bath. To each bottle was then added 25 ml. of N/1 citric acid solution so that the resulting dilution represented N/4 strength; the contents of the bottles were then shaken, and subcultures taken at intervals. The results were read after 48 hours' incubation by noting the turbidity of the tubes in which growth had taken place.

The results are given in table 1, from which it will be seen that the length of time required for disinfection is not in proportion to the number of bacteria present, in variations between 10 to 1,000 millions per ml.

We next tested the theory that clumps were responsible for the variation in results, having noted that, at times, pellicles were present on the surface of the 24-hour cultures. The filtered culture that was used for inoculating the water to be disinfected being quite turbid, we modified the technic in the next experiment by centrifuging the culture at moderate speed for from one half to one hour, or until it was clear. We employed 1 and 3 ml. of centrifuged culture for the various tests. Our aim was to present single bacterial bodies to the action of the dis-

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TABLE 1

RESULT OF EXPERIMENTS. NUMBER OF BACTERIA SEEMS TO HAVE NO DETERMINING EFFECT  
ON TIME REQUIRED FOR DISINFECTION

Millions per ml.	10.0	12.5	15.0	20.0	25.0	30.0	40.0	50.0	63.0	80.0	100.0	Min.
1000.0												
800.0								x				
630.0								x				
500.0						x						
400.0						x						
300.0							x					
250.0												
200.0						x						
150.0												
125.0					x					x		
100.0												
80.0												
63.0							x					
50.0						x				x		
40.0						x						
30.0												
25.0												
20.0												
15.0										x		
12.5												
10.0												

TABLE 2

DISINFECTION OF WATER INOCULATED WITH CENTRIFUGED CULTURE. AVERAGE DISINFECTING  
TIME 15 MINUTES

				x			
				x			
		x		x			
		x		x			
		x		x	x		
x	x	x		x			
x	x	x		x			
x	x	x		x			
x	x	x		x			
x	x	x		x	x		
5	10	15	25	40	60	Minutes	

infectant. The results shown in table 2, in which each x represents the end point in one experiment, show that centrifugalization lessens the disinfection time, but does not contribute to much greater constancy of results. That the lessening of the disinfection time was not due to numbers, was shown by the following facts: 1. No consistent differences were found between those experiments in which we used 1 ml. and those in which we used 3 ml. for inoculating the water. 2. A few counts showed the bacteria present in the water to be well above the lower count of our previous experiment.

TABLE 3  
DISINFECTION OF WATER INOCULATED WITH CULTURE CENTRIFUGED WITH COTTON. AVERAGE  
DISINFECTING TIME 25 MINUTES

			x			
			x			
			x			
			x			
			x			
			x			
	x	x	x			
	x	x	x			
	x	x	x			
	x	x	x			
	x	x	x	x	x	
	x	x	x	x	x	
5	10	15	25	40	60	Minutes

As it was found difficult to be absolutely certain that the clumps were entirely excluded in the experiments shown in table 2, owing to the fact that some of the sediment at the bottom of the centrifuge tube would tend to float upward into the liquid during the subsequent manipulations, we placed, in our next series of experiments, sterilized absorbent cotton in the bottom of the centrifuge tube, so as to entangle the sediment. This was quite successful as far as eliminating visible clumps was concerned. That this modification had no effect on constancy of results is shown in table 3. For the fact that, in this set of experiments, the disinfection time was actually somewhat lengthened—to an average of 25 minutes as compared with an average of 15 minutes in experiment 2—we have no explanation to offer.

## CONCLUSIONS

Within the limits of the observations made, neither variations in the numbers of bacteria nor the presence of clumps are the determining factors for variations in results in observations on disinfecting time.

The reason for these variations has not been determined.

To command confidence in results in experiments of this kind, a much larger number of observations is required than are usually made; the average might then be noted, and only large differences in disinfection time considered conclusive evidence of difference between different agents.